# Protocol of RT-PCR

## Kit Contents:

5×gDNA Buffer

FQ-RT Primer Mix

RT Enzyme Mix

10×Fast RT Buffer

RNase - Free dd$H_2O$

Process:

Table 1. gDNA Clean-up Reaction Components

| Component | Volume/Reaction |
|---|---|
| 5× gDNA Buffer | 2 μl |
| Total RNA | - |
| RNase-Free dd$H_2O$ | Up to 10 μl |

Table 2. Reverse-Transcription Reaction Components

| Component | Volume/Reaction |
|---|---|
| 10× Fast RT Mix | 2 μl |
| RT Enzyme Mix | 1 μl |
| FQ-RT Primer Mix | 2 μl |
| RNase-Free dd$H_2O$ | Up to 10 μl |

1. Thaw template RNA on ice. Thaw 5×gDNA Buffer、FQ-RT Primer Mix、10×Fast RT Buffer、RNase-Free dd$H_2O$ at room temperature (15-25°C), then place on ice immediately after thawing. Mix each solution by vortex, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare a fresh master mix to clean up genomic DNA according to Table

1. Mix thoroughly and carefully by vortex for no more than 5 s. Centrifuge briefly to collect residual liquid from the sides of the tube, incubate the mixture for 3 min at 42°C then store on ice.

3. Prepare a fresh master mix for revise transcription according to table 2. Mix thoroughly and carefully by vortex for no more than 5 s. Centrifuge briefly to collect residual liquid from the walls of the tube.
4. Add revise transcript mixture into the liquid getting from step 1, mix thoroughly.
5. Incubate for 15 min at 42°C.
6. Incubate for 3 min at 95°C, then store on ice. cDNA accepted could be used in the following experiments or stored in low temperature.